

Eotaxin, but not IL-8, is increased in upper and lower airways of allergic rhinitis subjects after nasal allergen challenge

Aleksandra Semik-Orzech, M.D., Ph.D.,¹ Adam Barczyk, M.D., Ph.D.,¹ Ryszard Wiaderkiewicz, Ph.D.,² and Władysław Pierzchała, M.D., Ph.D.¹

ABSTRACT

The aim of this study was to assess the impact of a single nasal allergen challenge (NAC) on levels of eotaxin and IL-8 and the inflammatory cells in upper and lower airways of allergic rhinitis (AR) patients. Twenty-four AR patients and 12 control subjects entered a sequential nasal placebo challenge and NAC study, out of the pollen season. Nasal lavage fluid (NLF) was obtained at baseline, 15 minutes, and 1, 5, and 24 hours postchallenge. Before and 24 hours after placebo/allergen challenge induced sputum was performed. NLF and induced sputum were evaluated for total cell count (TCC) and differential cell count and analyzed for concentrations of eotaxin and IL-8 using ELISA method. NAC in AR subjects was associated with significantly increased sputum ($p = 0.008$) and NLF ($p < 0.001$) eotaxin levels. Post-NAC IL-8 levels were significantly increased in NLF ($p < 0.0001$) but not in sputum ($p = 0.080$) of AR subjects. Increased eotaxin levels in NLF positively correlated with the increased TCC and eosinophils. Positive correlations were also found between NLF increased eotaxin level and sputum TCC, eosinophils, and macrophages. NAC is associated with the increased levels of eotaxin in lower airways of AR subjects. Allergen-induced secretion of eotaxin in nasal mucosa of AR subjects is involved in determining the cellular character of both upper and lower airway inflammation.

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Progress made in recent years has brought about some great changes in the understanding of allergic disorders pathophysiology. It has become clear that allergic processes in upper and lower parts of the respiratory tract are characterized by similar triggers, inflammatory cells mediators and treatment modalities.^{1,2} This has led to the development of the “integrated airway hypothesis,” which regards allergic diseases of upper and lower airways as different facets of a generalized, common inflammatory process.^{3,4} Studies conducted in allergic rhinitis (AR) patients without asthma or bronchial hyperresponsiveness revealed that nasal allergen challenge (NAC) led to the increased number of eosinophils as well as enhanced expression of ICAM-1, VCAM-1, and E-selectin on microvasculature of both nasal and bronchial biopsy specimens.⁵ In a study by Beeh *et al.*⁶ NAC in nonasthmatic AR subjects led to increased postchallenge sputum levels of eosinophil cationic protein and sICAM and decreased levels of IL-10. In our former study we observed increased levels of IL-17 and RANTES in sputum of AR

patients after NAC.⁷ The results of these studies reinforce the notion that nasal exposure to allergen up-regulates not only upper but also lower airway inflammatory response, suggesting that the direct contact between allergen and mucosa is not necessary to elicit allergic inflammation.

Data obtained in human atopic subjects give strong evidence for the role of eotaxin in mediating eosinophil recruitment in AR.^{8,9} In⁹ Terran *et al.* showed that NAC in AR patients led to parallel increases of eosinophil count and eotaxin levels in nasal lavage fluid (NLF). The concentration of eotaxin in lavage samples showed strong correlation with eosinophil count. In the same study eotaxin was shown to have the most potent effect among various eosinophil chemoattractants.⁹

IL-8 is a powerful chemotactic stimulant for both neutrophils¹⁰ and primed eosinophils.¹¹ Data regarding the impact of allergen exposure on IL-8 levels in upper airway in AR are conflicting. In study by Kuna *et al.*¹² levels of IL-8 in nasal secretions of AR patients during pollen season were significantly decreased when compared with its levels out of season. However, in other studies, levels of this chemokine have either not changed^{13,14} or increased^{15,16} after allergen exposure.

Because both eotaxin and IL-8 have proved to recruit cells to sites of allergic inflammation, we hypothesized that they might also be involved in the nasobronchial interactions after local allergen exposure. To test this

From the Departments of ¹Pulmonology and ²Histology and Embryology, Medical University of Silesia, Katowice, Poland

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Address correspondence and reprint requests to Aleksandra Semik-Orzech, M.D., Ph.D., Department of Pulmonology, Medical University of Silesia, Medyków 14, 40-752 Katowice, Poland

E-mail address: asemik@interia.pl

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hypothesis we assessed the impact of NAC on levels of both chemokines simultaneously in upper (NLF) and lower (induced sputum) airways of AR patients and compared them with healthy controls.

MATERIALS AND METHODS

Study Population

Sixty-two patients from the outpatient allergology unit of the Pulmonology Department, Silesian Medical University, were screened for AR symptoms. Of these, 24 fulfilled all entry criteria and were recruited. AR was diagnosed by a typical history (nasal obstruction, rhinorrhea, sneezing and postnasal drip during grass pollen season) for at least 2 consecutive years and positive skin-prick test (SPT) to grass pollen. Patients with concurrent allergy to tree pollen were excluded from the study to avoid priming of the nasal mucosa. Study participants were required to withhold any systemic or local medication that might affect the response to NAC at least 6 weeks before they entered the study. None of the patients had active AR during the study. Other exclusion criteria for entry were (1) age <18 and >35 years, (2) smoking history of >10 pack-years, (3) history of infection for 4 weeks before the test day and in course of the study, (4) gross anatomic anomalies or previous nasal/sinus surgery, (5) nonallergic nasal/sinus disease, (6) any chronic inflammatory processes or active allergic diseases other than AR, (7) history of specific immunotherapy in the past, (8) pregnancy, (9) forced expiratory volume in 1 second (FEV₁) of <80% of predicted values, and (10) poor patient compliance.

Twelve control subjects volunteered to take part in the study. They had no symptoms and no history of AR or any other chronic disease and did not use any local or systemic medication. SPT for a panel of common aeroallergens was negative in all subjects. Exclusion criteria were the same as for the AR patients.

Lung Function

Lung function was measured with dry spirometer (MasterLab, Jaeger, Germany).

Skin-Prick Test

SPT were performed in line with the European Academy of Allergology and Clinical Immunology recommendations¹⁷ on the volar part of the forearm, using standardized lancets with 1-mm tips. Standard battery of common allergens including grass and tree pollen, molds, feathers and house-dust mites (Allergopharma, Reinbeck, Germany) was used.

Study Design

The study was approved by Ethics Committee of the Medical Faculty, Silesian Medical University, Kato-

wice, Poland. During the first phase, all subjects were intranasally challenged with placebo (diluent solution of the allergen extract). Twenty-four hours before challenge, induced sputum was performed in each patient. On the challenge day, baseline total symptom score (TSS) and active anterior rhinomanometry (AAR) were determined, to choose the nostril with lower resistance values for the challenge. Next, patients underwent nasal lavage on the more patent nasal cavity, to reduce levels of preexisting cell-free mediators to a stable baseline and exclude subjects with nonspecific nasal reactivity. AAR and TSS were again evaluated. In the absence of an irritant nasal response to diluent solution (nasal airway resistance [NAR] increase of $\geq 30\%$ compared with baseline), 1 spray of the diluent solution was applied to the previously lavaged nostril at time point 0. Nasal mucosa reaction was then reassessed, based on NAR values, TSS, and nasal lavage, at 15 minutes and 1, 5, and 24 hours post-NAC. Twenty-four hours after placebo challenge, induced sputum was performed again. After a washout period of at least 30 days, specific NAC was performed, following the aforementioned procedures.

Nasal Provocation Test

NAC was performed in line with the recommendations of the German Society for Allergology and Clinical Immunology¹⁸ and European Academy of Allergology and Clinical Immunology guidelines.¹⁹ The study was performed in the pollen-free winter months, in a room with constant temperature and humidity. Subjects were submitted to provocation between 8 and 12 A.M. and all tests were performed by the same investigator. No topical decongestants were used in course of NAC. Clinical response (TSS) and NAR determined by AAR were used, to evaluate NAC results at fixed time points. The same batch of standardized, commercial nasal extract of grass pollen and the diluent solution of the allergen extract (Allergopharma) was used throughout the study. The allergen solution containing 5000 standard biological unit/mL (ger. Standard Biologische Einheit per milliliter) of grass pollen mixture was delivered through the nostril into the inferior turbinate region by a nasal pump sprayer insufflating 1 spray (0.04–0.05 mL) into the challenged nasal cavity. During and shortly after insufflation, the patient held their breath and kept their head in a forward prone position to avoid swallowing or aspiration of allergen into lower airways. NAC was considered positive when ipsilateral NAR value increased $\geq 60\%$ (compared with the value after baseline nasal lavage) and/or TSS was >3 points at 15 minutes post-NAC.¹⁸

Nasal Lavage

Nasal lavage was performed by the “nasal pool” technique described by Greiff and coworkers.²⁰ A 10-mL

syringe with closely fitting nostril filled with 8 mL of warm, sterile saline, was slowly instilled into the nasal cavity for 5 minutes. Then, by decompression, it was recovered into the device and immediately processed.

Sputum Induction and Analysis

Sputum was induced by hypertonic saline inhalation (3, 4, and 5% for 7 minutes) by an ultrasonic nebulizer device (Thomex MB, Medbryt, United Kingdom) and processed according to a method previously described.²¹

Measuring Cytokine Levels

Nasal and sputum eotaxin and IL-8 concentrations were measured by ELISA kits (R & D Systems, Minneapolis, MN) according to the manufacturer's protocol. The minimum detectable doses of eotaxin and IL-8 were 5.0 and 3.5pg/mL, respectively.

Statistics

Statistical evaluation was performed with software package (Statistica 6.0). Kolmogorov-Smirnov test was used to test variables for normal distribution. Student's *t*-test or Wilcoxon test was used to determine pre-/postchallenge differences. Nasal response to allergen was evaluated by Friedman ANOVA. If statistical significance was established, further analysis with Wilcoxon test was made. Intergroup comparisons were performed using Mann-Whitney *U* test. Spearman's rank correlation test was used to evaluate correlations between different parameters. Values of $p < 0.05$ were accepted as statistically significant.

RESULTS

Baseline characteristics of patients included is summarized in Table 1. Both groups were comparable with respect to age, sex, pretest FEV₁ values, and smoking status.

No adverse reactions and no significant changes in FEV₁ were noted after placebo/allergen administration at 15 minutes and 1 hour postchallenge. Rhinitis symptoms (TSS of ≥ 3) and/or $\geq 60\%$ NAR increase were present in all AR subjects at 15 minutes after specific NAC, but not after placebo challenge. Also, none of the control subjects have shown positive response to NAC either with allergen or placebo.

Induced Sputum Cytokine Levels

Measurable concentrations of eotaxin and IL-8 were found in all sputum samples assayed. Changes in sputum cytokine levels induced by NAC in both groups are presented in Table 2. Baseline sputum eotaxin (but not IL-8) concentrations were significantly higher in AR than in the control group ($p < 0.05$). Twenty-four hours postallergen (but not placebo) challenge, mean eotaxin level in induced sputum of AR subjects was

Table 1 Subjects demographics

	Allergic Rhinitis Patients	Control Group
Subject number (<i>n</i>)	24	12
Age (yr)	26.5 ± 3.2 (18–31)	25.9 ± 3.0 (21–30)
Sex (M/F)		
<i>n</i>	14/10	7/5
Percent	58.3/41.7	58.3/41.7
FEV ₁	108.9	105.70
Percent predicted	91.8–152.3	90.9–116.4
Wheal diameter to grass pollen in SPT	9.5	0.0*
mm	6.0–21.0	0.0–1.5
Cigarette smoking	21/1/2	10/0/2
N/Ex/Y(%)		

Data are expressed as mean values ± SD or medians and ranges.

* $p < 0.001$ for intergroup comparison (Mann-Whitney *U* test).

n = Subject number F = females; M = males; FEV₁ = forced expiratory volume in 1 s (percentage of predicted value); SPT = skin-prick test; N = nonsmokers; Ex = exsmokers; Y = current cigarette smokers.

significantly ($p = 0.008$) elevated, when compared with baseline (Fig. 1 A). There was a trend for elevated post-NAC IL-8 levels in induced sputum of AR subjects ($p = 0.08$; Fig. 1 B). Postallergen eotaxin (but not IL-8) sputum levels were also significantly ($p = 0.001$) higher in AR subjects, than in controls. Sputum eotaxin and IL-8 concentrations in control subjects remained unaffected ($p > 0.05$) both after allergen and after placebo challenge.

Induced Sputum Cellularity

Sputum cell viability did not differ between both groups ($p > 0.05$) and was higher than 76.5%. Compared with healthy controls, baseline induced sputum of AR subjects was characterized by higher absolute number of total cell count (TCC), eosinophils, neutrophils, and macrophages ($p < 0.05$, all intergroup comparisons). Nasal allergen (but not placebo) challenge in AR subjects induced further significant ($p < 0.001$) influx of eosinophils into the lower airways (Table 3). No changes in induced sputum cellularity were observed in the control group after both allergen and placebo challenge. When compared with healthy controls, induced sputum of AR subjects was characterized by higher absolute number of TCC, eosinophils, neutrophils, and macrophages both after placebo and after allergen challenge ($p < 0.05$, all intergroup comparisons).

Table 2 Sputum cytokine level changes induced by allergen and placebo challenge in allergic rhinitis subjects and healthy controls

	Allergy Rhinitis Patients				Control Group			
	Allergen Challenge		Placebo Challenge		Allergen Challenge		Placebo Challenge	
	Prechallenge	Postchallenge	Prechallenge	Postchallenge	Prechallenge	Postchallenge	Prechallenge	Postchallenge
Eotaxin pg/mL	297.5 0.0–1040.6	457.1* 178.5–1223.8	301.2 17.9–930.5	278.5 25.9–935.8	104.4 47.9–242.8	106.9 40.0–228.8	100.0 45.0–210.0	101.8 45.0–217.6
IL-8 pg/mL	16.3 4.0–266.2	20.0 7.0–327.6	10.5 3.2–78.9	11.8 3.9–49.3	20.3 9.0–38.2	23.0 11.0–40.6	21.1 8.0–41.8	24.3 7.6–43.0

Data are expressed as medians and ranges.

* $p < 0.05$ for pre-/postdifferences (Wilcoxon matched-pairs, signed-rank test).

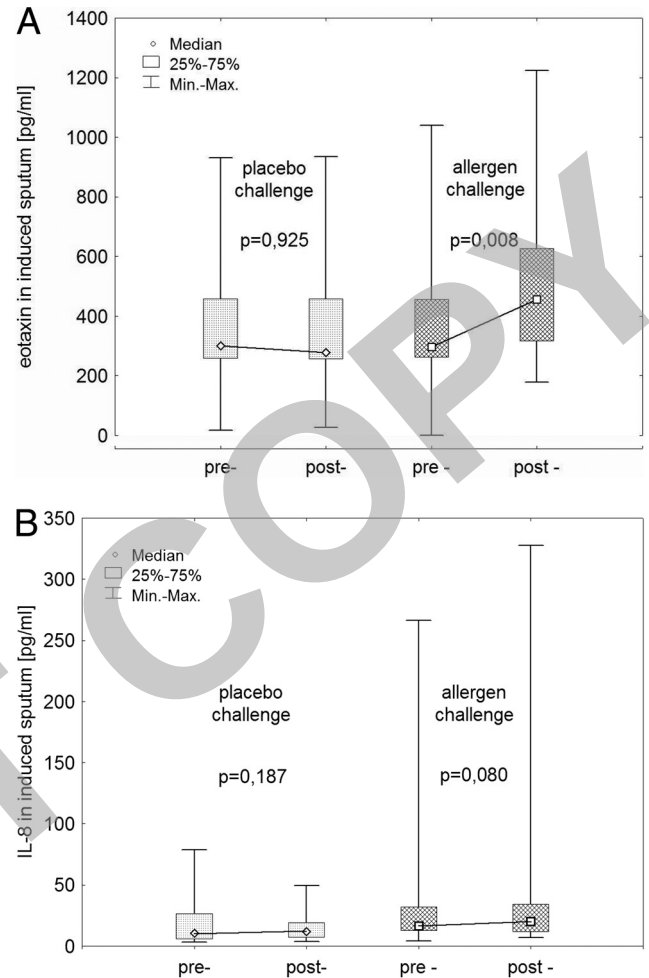


Figure 1. Time course of (A) eotaxin and (B) IL-8 in induced sputum of allergic rhinitis patients after nasal allergen and placebo challenge.

Nasal Lavage Cytokine Levels

The mean recovery of NLF was $68 \pm 9.4\%$. There were no significant differences in baseline eotaxin and IL-8 NLF concentrations between both groups ($p > 0.05$, both cytokines). In AR subjects NAC induced increase in eotaxin and IL-8 NLF levels when compared with baseline ($p < 0.001$, both cytokines; Fig. 2, A and B). Significant increase in NLF eotaxin level was noticed at 1 ($p = 0.030$), 5 ($p < 0.001$), and 24 hours ($p = 0.003$) post-NAC. Medians and ranges were as follows (pg/mL): baseline, 6.93 (1.28–10.36); 15 minutes, 2.79 (0.00–7.19); 1 hour, 7.60 (2.93–14.61); 5 hours, 14.20 (8.22–28.41); and 24 hours, 17.49 (3.21–31.38). When compared with baseline, IL-8 levels in AR subjects were significantly increased in NLF taken at 5 hours post-NAC ($p < 0.001$). Medians and ranges were as follows (pg/mL): baseline, 23.05 (6.68–379.46); 15 minutes, 18.87 (0.00–88.70); 1 hour, 25.47 (6.12–259.54); 5 hours, 88.49 (9.13–680.41); and 24 hours, 43.92 (4.44–333.27). Temporary decrease occurred in lavage eo-

Table 3 Sputum cellularity changes induced by allergen and placebo challenge in allergic rhinitis subjects and healthy controls

	Allergic Rhinitis Patients				Control Group			
	Allergen Challenge		Placebo Challenge		Allergen Challenge		Placebo Challenge	
	Prechallenge	Postchallenge	Prechallenge	Postchallenge	Prechallenge	Postchallenge	Prechallenge	Postchallenge
Total cell count	1.36	1.73*	1.70	1.33	0.91	0.95	0.75	0.70
$n^* 10^6$ /g of sputum	0.56–5.60	0.57–6.20	0.65–3.87	0.43–3.52	0.27–1.40	0.29–3.50	0.31–1.61	0.21–1.16
Eosinophils	0.05	0.08*	0.05	0.04	0.01	0.01	0.01	0.01
$n^* 10^6$ /g of sputum	0.00–0.5	0.00–0.75	0.00–0.55	0.00–0.29	0.00–0.03	0.00–0.02	0.00–0.02	0.00–0.02
Neutrophils	0.54	0.71	0.54	0.47	0.29	0.32	0.3	0.26
$n^* 10^6$ /g of sputum	0.13–2.68	0.15–3.17	0.12–1.29	0.09–1.21	0.05–0.48	0.07–1.07	0.09–0.58	0.07–0.52
Macrophages	0.80	0.91	0.89	0.90	0.55	0.57	0.43	0.48
$n^* 10^6$ /g of sputum	0.16–2.58	0.18–2.57	0.14–2.57	0.18–2.37	0.22–0.89	0.17–2.06	0.21–1.23	0.09–0.81
Lymphocytes	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00
$n^* 10^6$ /g of sputum	0.00–0.06	0.000–0.08	0.00–0.04	0.00–0.06	0.00–0.01	0.00–0.02	0.00–0.02	0.00–0.02

Data are expressed as medians and ranges.

* $p < 0.05$ for pre-/postdifferences (Wilcoxon matched-pairs, signed-rank test).

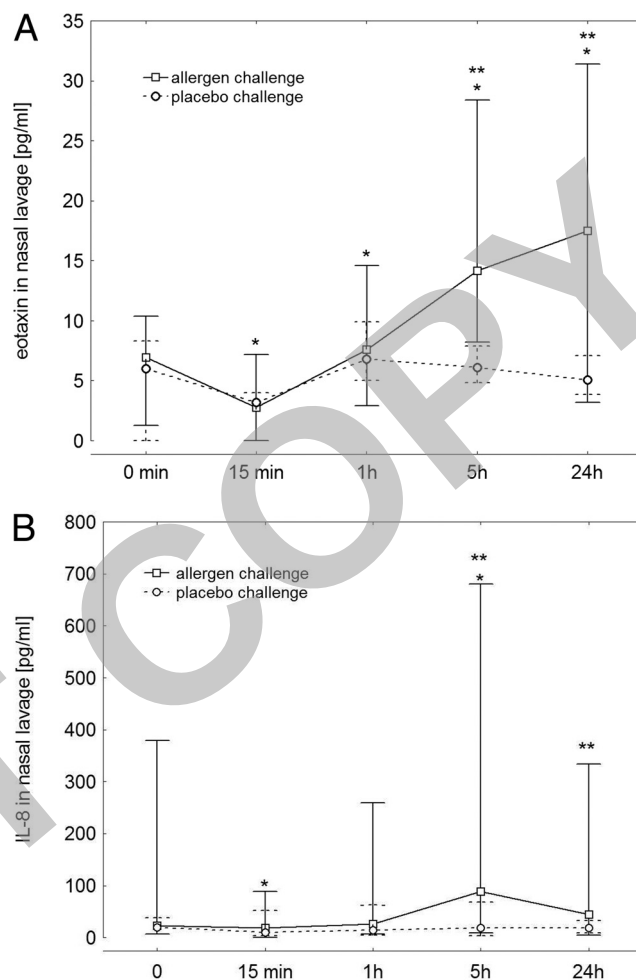


Figure 2. Time course of (A) eotaxin and (B) IL-8 in nasal lavage fluid of allergic rhinitis patients after nasal allergen and placebo challenge. The results are expressed as medians and ranges. * $p < 0.05$ compared with baseline value; ** $p < 0.05$ compared with post-placebo value.

taxin and IL-8 levels at 15 minutes after allergen and placebo administration in AR subjects and control group ($p < 0.05$), followed by a recovery at later time points (Fig. 2, A and B).

No significant increases were observed in either cytokine levels at any time point after placebo challenge in AR subjects, and in both after allergen and after placebo, in healthy subjects. The analysis of eotaxin concentrations in NLF samples revealed differences between both groups at all time points after allergen (but not placebo) challenge, with higher values obtained in AR subjects ($p < 0.05$, all time points). Similar differences in IL-8 NLF levels between both groups were noticed at 1, 5, and 24 hours post-NAC.

Nasal Lavage Cellularity

Cellular findings in NLF induced by NAC in AR subjects and controls are presented in Table 4. In AR patients NAC increased the number of TCC, including

Table 4 Nasal lavage cellularity induced by allergen and placebo challenge in allergic rhinitis subjects and healthy controls

	Allergen Challenge					Placebo Challenge					<i>p</i> *	
	Prechallenge	15 min	1 hr	5 hr	24 hr	<i>p</i> *	Prechallenge	15 min	1 hr	5 hr		24 hr
Allergic rhinitis patients												
Total cell count	0.08	0.25**	0.94**	2.09**	0.96**	<0.001	0.18	0.00**	0.00	0.22	0.20	<0.001
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.54	0.00–1.25	0.42–4.06	0.77–3.88	0.00–1.54		0.00–0.45	0.00–0.19	0.00–0.31	0.00–0.50	0.00–0.77	
Eosinophils	0.00	0.03**	0.40**	1.44**	0.24**	<0.001	0.00	0.00	0.00	0.00	0.00	NS
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.16	0.00–0.45	0.08–3.34	0.45–3.40	0.00–0.71		0.00–0.10	0.00–0.05	0.00–0.05	0.00–0.10	0.00–0.19	
Neutrophils	0.06	0.25	0.46**	0.61**	0.59**	<0.001	0.15	0.00**	0.00**	0.19	0.19	<0.001
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.47	0.00–0.74	0.15–3.41	0.23–1.10	0.00–1.08		0.00–0.45	0.00–0.19	0.00–0.22	0.00–0.47	0.00–0.44	
Monocytes	0.00	0.00	0.00	0.02	0.01	NS	0.00	0.00	0.00	0.00	0.02	NS
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.13	0.00–0.23	0.00–0.28	0.00–0.24	0.00–0.42		0.00–0.18	0.00–0.01	0.00–0.09	0.00–0.18	0.00–0.20	
Control group												
Total cell count	0.29	0.00**	0.00**	0.25	0.25	<0.001	0.27	0.00**	0.00**	0.24	0.25	<0.001
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.63	0.00–0.00	0.00–0.00	0.00–0.63	0.00–0.50		0.00–0.36	0.00–0.00	0.00–0.00	0.00–0.42	0.00–0.63	
Eosinophils	0.24	0.00**	0.00**	0.22	0.22	<0.001	0.25	0.00	0.00	0.20	0.22	0.001
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.45	0.00–0.00	0.00–0.00	0.00–0.48	0.00–0.45		0.00–0.35	0.00–0.00	0.00–0.00	0.00–0.42	0.00–0.63	
Neutrophils	0.00	0.00	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00	0.00	NS
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.02	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00		0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	
Monocytes	0.03	0.00**	0.00**	0.00**	0.01	0.002	0.03	0.00**	0.00**	0.00**	0.02	0.008
<i>n</i> * 10 ⁵ /mL of lavage	0.00–0.23	0.00–0.00	0.00–0.00	0.00–0.14	0.00–0.11		0.00–0.10	0.00–0.00	0.00–0.00	0.00–0.10	0.00–0.12	

Data are expressed as medians and ranges.

*The p value for differences in repeated measurements (Friedman ANOVA test).

**p < 0.05 for pre-/postdifferences (Wilcoxon matched-pairs, signed-rank test).

NS = nonsignificant.

eosinophil and neutrophil count ($p < 0.001$, all comparisons). Fifteen minutes post-NAC, AR subjects presented significant influx of eosinophils into NLF ($p < 0.05$) and the eosinophil count was still increased at 1, 5, and 24 hours after the challenge ($p < 0.001$, all comparisons). An increase in neutrophil count over the baseline at 1, 5, and 24 hours postchallenge was also noticed ($p < 0.001$, all comparisons). The peak influx of both cell types occurred at 5 hours post-NAC. The diluent challenge in AR subjects did not increase TCC at any time point, rather a significant ($p < 0.05$) decrease occurred at 15 minutes and 1 hour (TCC and neutrophils). TCC, neutrophil, and macrophage number was also decreased ($p < 0.05$) in NLF taken from control subjects at 15 minutes and 1 hour after specific and placebo challenge. The difference between allergen and diluent challenge in AR subjects with respect to eosinophil and neutrophil influx was significant at 15 minutes and 1, 5, and 24 hours post-NAC ($p < 0.05$, all comparisons). Control subjects showed no increase in cell numbers compared with baseline both after allergen and after placebo challenge. In postallergen NLF samples of AR subjects, the increase of TCC, eosinophil, and neutrophils was significant when compared with control group at all time points post-NAC ($p < 0.001$, all comparisons).

Correlations between Inflammatory Markers in Upper and Lower Airways

Baseline findings in correlation analysis were related to the peak cytokine levels observed. In postchallenge NLF samples from AR subjects, positive correlations occurred between eotaxin level at 5 hours, eosinophil count at 5 ($r = 0.583$; $p = 0.031$) and 24 hours ($r = 0.378$; $p = 0.026$), and TCC at 24 hours ($r = 0.483$; $p = 0.030$). NLF eotaxin level at 5 hours post-NAC correlated positively with the post-NAC sputum cell counts: TCC ($r = 0.511$; $p = 0.021$), eosinophils ($r = 0.555$; $p = 0.011$), and macrophages ($r = 0.452$; $p = 0.045$). Increased sputum and NLF eotaxin levels at 24 hours post-NAC were also positively correlated ($r = 0.551$; $p = 0.024$). No correlations were found between increased sputum eotaxin levels and sputum eosinophil count in AR subjects 24 hours after NAC.

DISCUSSION

AR is an inflammatory disorder in which tissue recruitment of inflammatory cells along with their directed migration under the influence of chemokines are well defined.²² There is increasing evidence for close links between upper and lower respiratory tracks, including common mechanisms and clinical interactions.^{1-4,23} Most experimental studies on chemokine profile in AR have focused on the upper part of the airway, without studying rhinitis in the context of

lower airway inflammation. With this in mind, we evaluated the possible involvement of eotaxin and IL-8 in the granulocyte influx into both nasal and bronchial lumen after NAC.

To our best knowledge, this is the first demonstration of elevated sputum eotaxin concentrations in AR subjects after NAC. Although not significant, we observed the trend to elevated IL-8 in the lower airway of AR subjects after NAC, suggesting that enrollment of a larger cohort of AR subjects would be likely to reveal statistically positive findings.

Several studies have stressed that recruitment and activation of eosinophils is controlled by the release of chemotactic agents such as eotaxin.⁸ High concentrations of eotaxin are present in sputum²⁴ and bronchoalveolar lavage fluid (BALF)²⁵ of asthmatic patients together with the increased expression of eotaxin mRNA and protein in lower airway epithelial cells.²⁵ Increased eotaxin levels that correlated with increased eosinophil count were found in BALF of allergic asthma patients after segmental bronchial provocation.²⁶ In a study by Li *et al.*,²⁷ where murine models of pure upper airway allergic inflammation were developed, levels of eotaxin and eosinophil count after NAC were significantly elevated in NLF and serum, but not in BALF. In our study increased eotaxin levels and eosinophil count were found after NAC in the bronchial lumen of AR patients. Eosinophilic bronchitis in nonasthmatic AR subjects have already been established by sputum analysis^{7,28} and bronchial biopsy,^{5,29} indicating that allergen exposure is a trigger of lower airway eosinophilia. However, our study for the first time highlights the importance of eotaxin in the development of lower airway eosinophilic inflammation after NAC in nonasthmatic AR subjects. Increased sputum eotaxin level may reflect the enhanced local production of this cytokine after allergen exposure. Another possibility would be that plasma exudation may move eotaxin already present in the bronchial wall into the airway lumen by its binding and transporting properties.³⁰ Because we found no positive correlation between increased sputum eotaxin level and eosinophil count, we lack direct proof that involvement of eotaxin present in lower airways is one of the principal mechanisms leading to bronchial infiltration of these cells in AR. We suspect that the single time point of 24 hours after NAC might not have been sufficient to detect such correlation. However, given the fact that increased sputum eosinophil count found in our former study⁷ was associated with increased sputum levels of IL-17 and RANTES, it is also possible that eosinophilic bronchitis in AR is determined by the combination of various cytokines acting together or in a sequence.

The results of our study give strong evidence for the role of eotaxin in mediating eosinophil recruitment into upper airways, as suggested by other investiga-

tors. Previous studies revealed that intranasal administration of eotaxin induced significant influx of eosinophils into both unprimed³¹ and allergen-primed³² nasal mucosa of AR patients. In AR, up-regulation of eotaxin mRNA³³ and protein^{33,34} in nasal mucosa have been detected. In some⁹ but not all³⁵ studies, NAC in AR subjects led to parallel increases of eosinophil counts and eotaxin concentrations in NLF where both parameters were strongly correlated. Our findings are in agreement with most of the aforementioned studies, indicating that eotaxin plays an important role in eosinophil-dependent nasal mucosa inflammation in AR. NLF eotaxin levels in our study showed a typical late-phase kinetic decreasing below the baseline level at 15 minutes (which is likely a washout effect of sequential lavages at short time intervals), and then slowly increasing at 1 hour and reaching its maximum levels at 5 hours post-NAC. Consistent with the results of Erin *et al.*,³⁶ the influx of inflammatory cells into nasal secretions in our study was prominent during the late phase of allergic reaction and the maximum eotaxin levels were also detected at this period, implicating that the contribution of eotaxin is particularly important for the late recruitment of inflammatory cells into nasal mucosa after NAC. In our study, increased NLF eotaxin levels positively correlated with the postchallenge sputum eotaxin levels, as well as with sputum eosinophil count and TCC, suggesting the relevance of the secretion of eotaxin in the upper airways to the bronchial eosinophil recruitment. The results of former studies in which eosinophil count in bronchial lumen of AR subjects remained unchanged despite the increased post-NAC serum (but not sputum) levels of IL-5⁶ and eotaxin²⁷ suggested that only locally active chemokines are involved in the trafficking of eosinophils to the bronchial wall after NAC. In view of these studies, our data are of interest, because they provide further evidence of its potential importance in naso-bronchial interactions in AR.

IL-8 is a potent neutrophil, monocyte, and T-lymphocyte chemotactic stimulant that is reported to play an important role in the late phase of allergic reaction through the induction of chemotaxis of eosinophils primed by IL-3 or granulocyte macrophage colony-stimulating factor.¹¹ To our best knowledge, this is the first study that investigated IL-8 concentrations in lower airways of AR patients after NAC. Although not significant, there was a trend to the elevated sputum levels of this chemokine suggesting that the number of patients enrolled might not have been sufficient to reveal statistically positive findings. However, the obtained results agree with the results of Lopuhaa *et al.*³⁷ who found that levels of IL-8 and neutrophil count in induced sputum of nonasthmatic AR patients remained unchanged 24 hours after BAC. Unless verified on a larger cohort of AR patients, the findings of the

current study and a study by Barczyk *et al.*²¹ suggest that IL-8 and neutrophils contribute more to bronchial inflammation in allergic asthma than in AR.

In the current study IL-8 showed a similar release pattern as in other studies^{38,39} with decreased levels 15 minutes post-NAC (which is likely a washout effect) and significantly increased secretion at 5 hours post-challenge. The role of IL-8 in the pathogenesis of AR is controversial. NAC with recombinant IL-8 caused an influx of neutrophils in AR subjects.⁴⁰ Although increased IL-8 levels were noted in nasal secretions of AR subjects both during the pollen season^{41,42} and after NAC,^{15,16,39} some observations suggested that this chemokine does not play an important role in AR. Several authors reported that natural allergen exposure does not lead to significant changes of IL-8 in nasal secretions,^{14,43} while other authors reported its decreased levels during the season.^{12,13} These conflicting findings may be related to the differences in chemokine regulation between natural exposure and NAC, depending on the magnitude and continuity of exposure to allergen. It is possible that single exposure of unprimed nasal mucosa to relatively high doses of allergen in a course of NAC causes temporary up-regulation of IL-8. On the contrary, during the pollen season prolonged exposure to variable allergen concentrations might be associated with the down-regulation of IL-8 levels as well as the number and activity of neutrophils on the mucosal surface.¹³

CONCLUSIONS

Eosinophilic bronchitis in nonasthmatic AR patients is associated with the increased sputum eotaxin concentrations. Enhanced nasal production of eotaxin (but not IL-8) in nonasthmatic AR patients is involved in determining the eosinophilic character of not only nasal but also bronchial inflammation during the late phase of allergic reaction.

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